Thermal inactivation of *Clostridium perfringens* vegetative cells in ground beef and turkey as affected by sodium pyrophosphate

Introduction

Clostridium perfringens is an important cause of food-poisoning world-wide (Dische and Elek 1957, Centers for Disease Control 1985, Stringer et al. 1980). Outbreaks primarily involve meat and meat products; the illness occurs after ingestion of approximately 10^6–10^7 viable cells per gram of food (Hauschild 1970). The ingested cells sporulate in the intestine and release a heat-labile enterotoxin known as *C. perfringens* enterotoxin, which is responsible for typical symptoms (Stark and Duncan 1971, Duncan 1975).

Phosphates may be used in processed meat and poultry products to assure microbiological safety (Kijowski and Mast 1988). The primary functions of phosphates in meat products are to: protect flavor; bind structured meats; and retain moisture and thus increase yield. Choi et al. (1987) reported that a 0·5% blend of phosphates may be added to

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reduce salt levels from 3 to 1·5% in frankfurters without processing or storage difficulties.

In response to consumer demands for fresh-tasting, high-quality, convenience meals that require minimal preparation time, there has been an increased production of ready-to-eat, extended shelf life refrigerated foods in the domestic and foreign markets. While the mild heat treatment given to such products kills the vegetative cells of spoilage and pathogenic bacteria, spores of *C. perfringens* which have a decimal reduction (*D*) value at 99°C of 17·3 to 19·8min can be expected to survive (Juneja and Majika 1995); the activated spores germinate, outgrow and multiply in cooked meat and poultry if rate and extent of cooling are not sufficient. Inadequate cooling practices have been attributed as a major cause of food-poisoning with *C. perfringens* (Bean and Griffin 1990). In the 15-year period, 1973–1987, beef and turkey were the vehicles for 26·8% (51/190) and 10% (19/190), respectively, of the *C. perfringens* outbreaks; the organism accounted for 10·2% of the total bacterial foodborne outbreaks (190/1869) and 11·2% of the total cases of bacterial foodborne disease (12234/108806) in the USA (Bean and Griffin 1990). Todd (1989a, b) estimated that 652000 cases of foodborne *C. perfringens* illness occur each year in the USA with an average of 7·6 deaths and annual costs of $123 million.

Juneja et al. (1994b) observed that *C. perfringens* spores germinated and grew from an inoculum of approximately 1·5$\log_{10}$ to about 6·0$\log_{10}$cfu g^{-1} when the cooling time to achieve 7·2°C was extended to 18h. These authors reported that pasteurized cooked beef must be cooled to 7·2°C in 15h or less to prevent growth from spores of *C. perfringens*. If the product does not receive sufficient heat to kill spores, and the cooling rate after heating is too slow, vegetative cells produced owing to growth from spores of *C. perfringens* must be killed by sufficient re-heating before consumption to guard against food-poisoning. Lack of data on the thermal-death-time values for *C. perfringens* vegetative cells in beef and turkey that included sodium pyrophosphate (SPP) in the formulation prompted us to define the heat treatment required to give a specified lethality for *C. perfringens* vegetative cells in these products. Accordingly, we quantitatively assessed the influence of SPP in beef and turkey on thermal inactivation of *C. perfringens* vegetative cells. The *D*- and *z*-values from this study could be used to establish re-heating temperatures that would minimize the potential danger of *C. perfringens* food-poisoning.

Materials and methods

Bacteria

Three strains of *C. perfringens*, NCTC 8238 (Hobbs serotype 2), NCTC 8239 (Hobbs serotype 3), and NCTC 10240 (Hobbs serotype 13), from the Eastern Regional Research Center culture collection, were used in this study. Stock cultures were maintained at 4°C in cooked-meat medium (Difco Laboratories, Detroit, Michigan).

Preparation of inoculum

To prepare inocula, 0·1ml of the stock cultures was individually transferred to 9·9ml of freshly prepared fluid thioglycollate medium (FTM, Difco). The inoculated medium was then heat shocked at 75°C for 20min and incubated at 37°C for 6h. Approximately 1ml of the 6-h culture was transferred to 9ml of FTM and incubated for 18h. The cells were harvested by centrifugation at room temperature for 10min at 7700×g and the cell pellet was washed twice and finally resuspended in sterile 0·1% peptone water (w/v) to a target level of 9$\log_{10}$cfuml^{-1}. The population densities in each inoculum suspension were enumerated by spiral plating (Spiral Biotech, Bethesda, Maryland; Model D) appropriate dilutions (in 0·1% peptone water), in duplicate, on Tryptic soy agar (TSA; Difco) plates to determine the initial number of bacteria. Equal volumes of each culture were combined in a sterile test tube to obtain a three-strain mixture of *C. perfringens* (9$\log_{10}$cfu ml^{-1}) prior to inoculation of meat.

Sample preparation, and inoculation

Raw 90% lean ground beef and lean ground turkey were obtained from a local retail market and frozen (-5°C) until use (approximately 60 days). After thawing at 4°C for 24h, the meat was placed in a thin layer on plastic trays and autoclaved at 121°C for 15min. The fat was poured off while the meat was hot and the meat was then cooled at 4°C to an internal temperature of 25°C. Sodium pyrophosphate was mixed into all ground beef and turkey samples with a Hobart mixer to give a final concentration of 0·15 or 0·3% (w/w). The pH of the ground beef was determined with a combination electrode (Sensorex, semi-micro, A. H. Thomas, Philadelphia, Pennsylvania) attached to an Orion model 601A pH meter. Duplicate 3-g ground beef or turkey samples were aseptically weighed into 15 x 22·9cm sterile whirl-pak sampling bags (Model B736, NASCO Modesto, California) and inoculated with 0·1ml of an appropriate dilution of *C. perfringens* cocktail so that the final concentration of cells was approximately $8 \log_{10} \text{cfug}^{-1}$. Negative controls included bags containing meat samples inoculated with sterile 0·1ml of 0·1% (w/v) peptone water. Thereafter, the bags were manually mixed to ensure even distribution of the organisms in the meat sample, compressed into a thin layer (approximately 1–2mm thick) by pressing against a flat surface, excluding most of the air, and then heat sealed.

Thermal inactivation and enumeration

Two replications were performed for each phosphate level in beef and turkey and the control samples (with no added SPP). Bags at room temperature were placed in a basket and then fully submerged in a temperature-controlled water bath (Exacal, Model Ex-251HT, NESLAB Instruments, Inc., Newington, New Hampshire) stabilized at 55, 57·5, 60, 62·5 or 65°C. The temperature was continuously monitored by two copper–constantan thermocouples inserted, prior to heat sealing, at the center of two uninoculated bags. The thermocouple readings were measured and recorded by a Keithyl-Metrabyte data logger Model DDL 4100 (Tauton, Maine) connected to a microcomputer. The thermocouple signal was sampled every second, and the two readings were averaged to determine the bag internal temperature. Come-up times, which were negligible, were included as part of the total heating time when these were used to calculate the *D*-values. Two bags for each replicate were then removed at designated time intervals; sampling frequency was based on the heating temperature. After removal, bags were immediately plunged into an ice-water bath, then analyzed within 30min. For determination of the number of surviving bacteria, sterile 0·1% peptone (3ml) was combined with each meat sample to obtain 1:1 (w/v) slurry and pummeled for 1min with a Stomacher 400 Lab-blender (Tekmar, Cincinnati, OH). Decimal serial dilutions were prepared in 0·1% peptone water and appropriate dilutions were surface plated, in duplicate, on to agar dishes containing Tryptose–sulfite–cycloserine (TSC) agar without egg-yolk enrichment (Hauschild and Hilsheimer 1974) using a spiral plater (Model D, Spiral Biotech, Bethesda, Maryland). Uninoculated samples were plated as controls. When increased sensitivity was required, 0·1ml of undiluted suspension was surface plated. The TSC agar plates were overlaid with an additional 10ml of TSC agar. After the overlaying, the agar was allowed to solidify before the dishes were placed into anaerobic jars. Surviving vegetative cell counts were determined after 48h of incubation at 37°C in a GasPak system (Baltimore Biological Laboratory, Cockeysville, Maryland). For each replicate experiment performed in duplicate, an average cfug^{-1} of four platings of each sampling point was used to determine the *D*-values.

Determination of D*-values*

D-values (time for 10-fold reduction in viable cells), expressed in minutes, were determined by plotting the $\log_{10}$ number of survivors against time for each heating temperature using Lotus 1-2-3 software. The line of best fit for survivor plots was determined by regression analysis (Ostle and Mensing 1975);

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(1981) grew *C. perfringens* in autoclaved ground beef at 37°C before thermal inactivation studies. The authors reported that *C. perfringens* vegetative cells of strains NCTC 8238 and NCTC 8798 had mean *D*-values at 57°C of 7·33 and 11·1 min, respectively; the values were 2·3 and 3·1 min at 59°C. Differences in *D*-values may be due to strain variation, recovery conditions (including the composition and pH of the medium), the presence of inhibitors, time and temperature of incubation and, above all, the heating menstruum. In addition, Roy et al. (1981) quantified *C. perfringens* destruction in autoclaved ground beef containing 17 and 22% fat in screw-capped test tubes, whereas we studied destruction of the organism in lean ground beef. In the present study, a higher recovery of heated *C. perfringens* cells was seen in beef. Compared with turkey, the increased thermal resistance of *C. perfringens* in beef may be attributed to the difference in properties of the two meat products, i.e., the effect of different meat species and the differences in fat content between the substrates.

The Gram-positive bacteria are generally more susceptible to phosphates than Gram-negative bacteria (Davidson and Juneja 1990). Kelch and Buhlmann (1958) tested commercial mixtures of tetrasodium pyrophosphate, sodium acid pyrophosphate, sodium tripolyphosphate, and sodium hexametaphosphate with or without heat against Gram-positive bacteria. In that study, while susceptibility of *Staphylococcus aureus* and *Streptococcus faecalis* was variable in nutrient medium, the organisms were completely inhibited by the phosphates in combination with heat (50°C). In the present study, increased heat lethality of *C. perfringens* cells in the presence of sodium pyrophosphate may be attributed to the additive or synergistic effect of sodium pyrophosphate and heat.

Thermal-death-time curves were plotted from *D*-values obtained in beef and turkey with and without SPP in order to calculate *z*-values. *Z*-values in beef, with and without added SPP, calculated from the curve between 55 and 62·5°C ranged from 6·22°C to 6·74°C (Fig. 1). These *z*-values do not agree with those reported by Roy et al. (1981). These authors reported *z*-values between 55 and

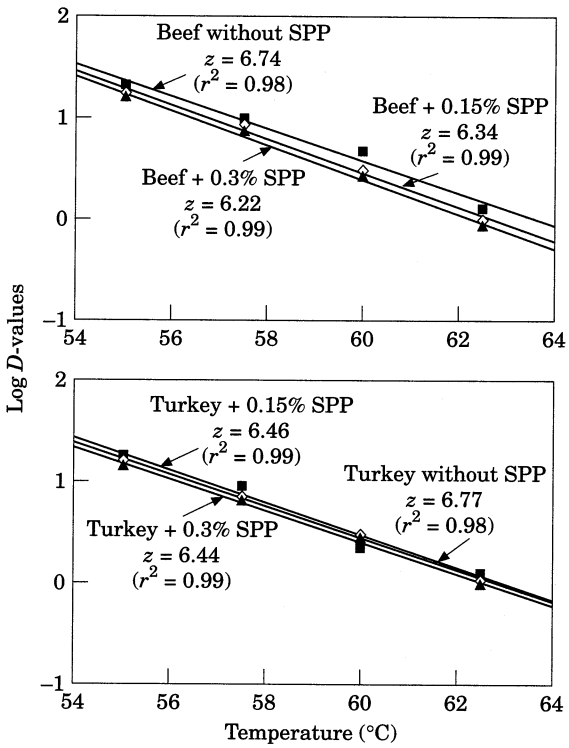

Figure 1. Thermal-death-time curves (*z*-values) for a three-strain mixture of *Clostridium perfringens* in precooked ground beef and turkey which contained 0, 0·15 or 0·30% sodium pyrophosphate, over the temperature range 55–62·5°C. *D*-values, used to determine the *z*-values, were the means of two replicates.

61°C that ranged from 3·1 to 3·8°C. In the present study, the *z*-values obtained in beef were very similar to that calculated from *D*-values obtained in the presence or absence of SPP in turkey; the values in turkey ranged from 6·44 to 6·77°C (Fig. 1).

Results from this study suggest that in precooked, refrigerated foods, inadequate re-heating temperatures may contribute to *C. perfringens* food-poisoning. When *C. perfringens* cells inoculated in beef ($8 \log_{10} \text{cfu g}^{-1}$) were heated at 65°C for 15 s, as many as $6 \log_{10} \text{cfu g}^{-1}$ of *C. perfringens* cells were recovered (Table 2). This could be overcome as heating at 65°C for 1 min completely inactivated $8 \log_{10} \text{cfu g}^{-1}$ of *C. perfringens* cells. Because SPP increased the sensitivity of cells to heat, heating for 30 s at 65°C inactivated the same number of cells. In turkey, regardless of the presence of SPP, heating at 60°C for 30 s was adequate to inactivate a high

number of cells. These results, with respect to re-heating temperature for inactivation for large numbers of *C. perfringens* cells, are in agreement with our earlier data (Juneja et al. 1994a). In that study, we reported that re-heating cooked beef to an internal temperature of 65°C before consumption would prevent food-poisoning because the vegetative cells were killed. The vacuum-packaged, precooked beef inoculated with *C. perfringens* cells was stored at 4°C for 7 days before the thermal-inactivation studies; in contrast, in the present study we inoculated precooked ground beef or turkey and assessed the heat resistance without prior storage. In the study by Juneja et al. (1994a), the storage at low temperatures may have resulted in induction of cold-shock protein and/or biochemical changes in bacterial membranes; however, the organisms' heat resistance was not altered. Willardsen et al. (1978) reported that inactivation of *C. perfringens* cells in ground beef began at approximately 55°C in a constantly rising-temperature (rate 8·5°C h^{-1}) water bath, but a minimal exposure to temperatures near 60°C may be insufficient to inactivate vegetative cells of *C. perfringens*. Smith et al. (1981) found that cooking beef in a water bath to an internal temperature of 60°C and holding for at least 12min reduced a population of *C. perfringens* cells by approximately three log cycles. Sundberg and Carlin (1976) reported that 1·6-kg rump roasts cooked in an oven at 107°C or 177°C or in an electric crock pot to internal temperatures above 77°C had counts of vegetative *C. perfringens* reduced by more than three log cycles. In another previous study, Bryan (1969) suggested a minimum temperature of 74°C for re-heating meat, but he later revised the internal temperature to 70°C (Bryan and McKinley 1974).

Control measures for *C. perfringens* food-poisoning must ensure that large numbers of vegetative cells are not consumed. Our study has shown that a significant reduction in vegetative cells of *C. perfringens* is obtained by re-heating precooked products to an internal temperature of 65°C for 1min immediately before consumption of the product. The data presented in Table 1 can be used as an aid to predict the time required at specified temperatures to achieve a certain number of log-cycle reductions of *C. perfringens* when heated in precooked beef or turkey, under the conditions described in the current study. Based on the thermal-death-time values determined in this study, contaminated cooked beef should be re-heated to an internal temperature of 62·5°C for at least 9·6min and turkey for 7·8min; this is based on the argument that re-heating temperature must destroy at least 6$\log_{10}$cfug^{-1} of *C. perfringens*. Also, the present study suggests that in the presence of 0·3% SPP in beef or turkey, 6min at 62·5°C would be sufficient to achieve a 6-*D* process. Thermal-death-time values from this study will assist restaurants and institutional food service settings in the design of acceptance limits on critical points that ensure safety against *C. perfringens* in cooked beef and turkey.

Table 2. Heat resistance[a] of *Clostridium perfringens* three-strain mixture in precooked ground beef and turkey that contained 0, 0·15 or 0·30% sodium pyrophosphate at 65°C

	Heating time (seconds)			
Heating menstruum	0	15	30	60
Beef without SPP[b]	8·1±0·1	6·5±0·1	1·4±0·3	ND[c]
Beef+0·15% SPP	8.1±0·3	5.8±0·2	ND	ND
Beef+0·3% SPP	7.9±0·2	5.2±0·1	ND	ND
Turkey without SPP	8.1±0·0	5.8±0·1	ND	ND
Turkey+0·15% SPP	8.0±0·4	4.1±0·5	ND	ND
Turkey+0·3% SPP	8.0±0·2	4.0±0·5	ND	ND

[a]Data (expressed as $\log_{10}$cfu g) shown are the means of two replicates, each performed in duplicate.
[b]Sodium pyrophosphate. [c]Not detected.

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